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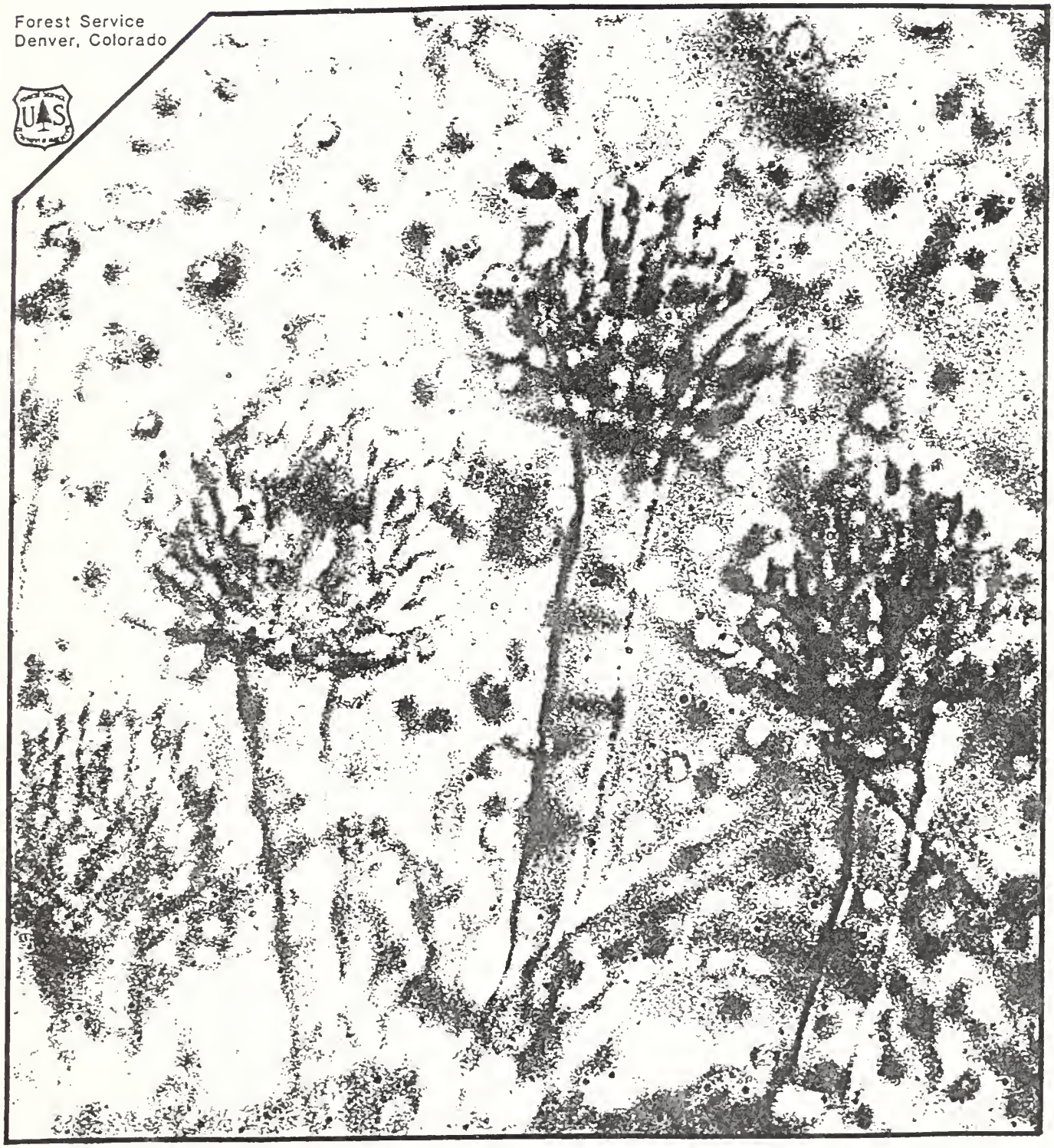
Soil Solar Heating and Thermotolerant Microorganisms Antagonistic to Fusarium spp.



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EFFECTS OF SOIL SOLAR HEATING ON THERMOTOLERANT MICROORGANISMS ANTAGONISTIC TO PATHOGENIC *FUSARIUM* SPP. AT BESSEY NURSERY, HALSEY, NEBRASKA

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ABSTRACT

In a field study of soil solar heating effects conducted during the summer of 1985, changes in population levels of *Fusarium* spp. and thermophilic/thermotolerant microorganisms were investigated at Bessey Nursery, Halsey, Nebraska. Solar heating reduced population levels of *Fusarium* spp., and increased population levels of the thermotolerant, *Aspergillus fumigatus*. Antagonism by thermophilic/thermotolerant microorganisms against *Fusarium* spp. pathogenic to conifer seedlings was tested. *A. fumigatus* and some thermotolerant bacteria isolated from nursery soil were strong competitors in culture against pathogenic isolates of *F. oxysporum, F. moniliforme,* and *F. acuminatum*. The competitive ability of thermotolerant microorganisms in culture implicates them in contributing to pathogen limitation due to solar heating, especially since most plant pathogens are not thermotolerant.

INTRODUCTION

Soil solar heating (solar pasteurization, soil solarization) is a cultural technique in which moist soil is covered with clear polyethylene for several weeks during the hottest months for control of soil-borne diseases (Katan et al., 1976). Solar heating has been effective against a variety of weeds and soil-borne pathogens, especially for agricultural crops in warm climates (Katan 1984). Solar heating has led to prolonged reductions in pathogen populations in some cases (Kassaby 1985). Proposed mechanisms for this effect include shifts in microbial populations (Greenberger et al., 1985).

Populations of Actinomycetales and thermophilic fungi sometimes increased after solar heating of California soils (Stapleton and DeVay, 1982), as did some gram-positive bacteria exhibiting antibiosis against *Geotrichum candidum* Link ex. Pers. (Stapleton and DeVay, 1984). Plant pathogens apparently are more sensitive to elevated temperatures than are saprophytes.

Previous studies in Colorado and Nebraska forest tree nurseries showed decreased populations of species of *Pythium*, *Fusarium*, and weeds due to solar heating (Hildebrand, 1985a and 1985b). The purposes of this study were to determine whether populations of thermophilic/thermotolerant microorganisms increased in solar heated Nebraska nursery soil, and whether some of these microorganisms were antagonistic to pathogenic *Fusarium* spp. Using the definition of Cooney and Emerson (1964), thermophilic microorganisms could not grow at 20°C, and had thermal optima above 40°C, while thermotolerant microorganisms could grow from at least 19°C through 50°C.

MATERIALS AND METHODS

Solar Heating

In summer 1985, study plots were established at the USDA Forest Service Bessey Nursery, located 3 km west of Halsey, Nebraska, at 838 m elevation. The soil in the study area was Meadin loamy sand of the Dunday Loup Association in the Middle Loup River Valley (Sherfey et al., 1965).

On June 21, the oat cover crop was plowed under for eight plots (3 x 5.5 m), four solar-heated and four untreated as controls. One Peabody Ryan model J thermograph was buried with its sensor at 15 cm in each of one solar and one control plots. In each plot, three soil samples (composites of ten cores taken to a depth of 15 cm with a soil

probe) were taken at 1.5 m intervals. The soil was irrigated to field capacity and 0.038 mm clear polyethylene sheeting laid on the solar plots, and the edges anchored with soil. After 45 days the polyethylene was removed and soil sampling repeated.

Assay for Microorganisms

All pre- and post-treatment samples were assayed for population levels of *Pythium* spp. and *Fusarium* spp. using the procedure of Johnson and Zak (1977), but with three plates of selective medium per sample instead of five. Significant changes in population levels were determined by the test for equality of means with unequal variances (Sokal and Rohlf, 1981).

For assay of thermophilic/thermotolerant microorganisms, samples from each plot were bulked to yield a total of four composite samples for each treatment. Serial dilutions of the composite samples were incubated at 50°C and 30°C in 8 percent nutrient broth with 2.5 percent glucose for two weeks. Population levels were estimated using the Most Probable Number (MPN) technique with five tubes per dilution (American Public Health Association, 1981).

Lower dilutions of the composite samples were incubated at 50°C on Emerson's yeast starch agar (YSA)(Stevens 1974), and YSA modified with 20 drops of 50 percent lactic acid and 4 ml of 5 percent streptomycin sulfate per 800 ml (MYSA). YSA plates (predominately Actinomycetales and other bacteria) were read after two days, while MYSA plates (fungi) were read after 11 days. Population levels were estimated by dividing the total number of colonies by the dilution factor.

Antagonism Tests

Fusarium spp., isolated from Colorado and Nebraska forest tree nursery soil and seedlings, were used in antagonism tests against thermophilic/thermotolerant microorganisms isolated from Bessey Nursery soil. The five isolates, three *F. oxysporum* (19, 25, and 26), *F. acuminatum* (23), and *F. moniliforme* (29), were pathogenic to lodgepole pine in previous tests (Hildebrand 1985b). These *Fusarium* isolates have been deposited at the Fusarium Research Center at Pennsylvania State University (for accession numbers see Table 4). All of the thermophilic/thermotolerant microorganisms tested against the *Fusarium* isolates could grow at 30°C in pure culture on YSA. *Fusarium* growth in antagonism tests was compared with that in pure culture and interactions observed.

For cross-streak tests, individual cells, conidia, and/or hyphal fragments were scraped from a thermophilic/thermotolerant culture with a sterilized inoculating loop and a single streak made on YSA plates. Plates were incubated for two days at 45°C, and then a 6-mm

plug (cut from a 2-week old culture with a cork borer) of *Fusarium* was placed adjacent to the streak for several plates for each of the five *Fusarium* isolates. Plates were then incubated at 30°C for two weeks. Thermophilic microorganisms used for cross-streaks included Actinomycetales, *Humicola grisea* Traaen var. *thermoidea* Coon. & Emers. (Figure 1a), *H. insolens* Coon. & Emers. (Figure 1b), and four thermotolerant bacterial isolates. The thermotolerant, *Aspergillus fumigatus* Fres. (Figure 1c), was streaked onto YSA and plugs of *Fusarium* inoculated the same day.

For the opposing-plugs test, plugs of thermophilic fungi were transferred to YSA plates, incubated at 45°C for two days, and then a plug of *Fusarium* was placed about 2.5 cm from the thermophile plug. Plates were then incubated at 30°C for two weeks. Thermophilic fungi used were one unidentified cleistothecial ascomycete, two deuteromycete (Moniliales) isolates, and *Penicillium dupontii* Griff. & Maublanc (Figure 1d). Plugs of two thermotolerant *Aspergillus fumigatus* isolates were transferred to YSA plates the same day as the *Fusarium*.

Penicillium dupontii (259) and *Aspergillus fumigatus* (268 and 269) have been deposited in the American Type Culture Collection (for accession numbers see Table 4).

RESULTS AND DISCUSSION

By the end of the solar-heating treatment (August 5, 1985), the polyethylene sheeting in the solar plots had some large tears, but was still in place. The control plots had extensive weed cover by this time. Due to the unusually cool, wet, and overcast conditions in July, temperatures under the polyethylene did not reach levels as high as in previous years (Hildebrand 1985b). Maximum temperatures under the polyethylene still averaged 8°C higher than in the control plot, but the highest temperature recorded in the solar plot was 40°C for a duration of 4 hours.

Populations of Microorganisms

Population levels of *Pythium* species were not changed by solar heating, but population levels of *Fusarium* species were significantly reduced (Table 1). Population levels (determined by MPN) of total microorganisms in both control and solar plots dropped over the summer (Table 2), possibly due to depletion of the food source from the cover crop which was plowed under early in the summer.

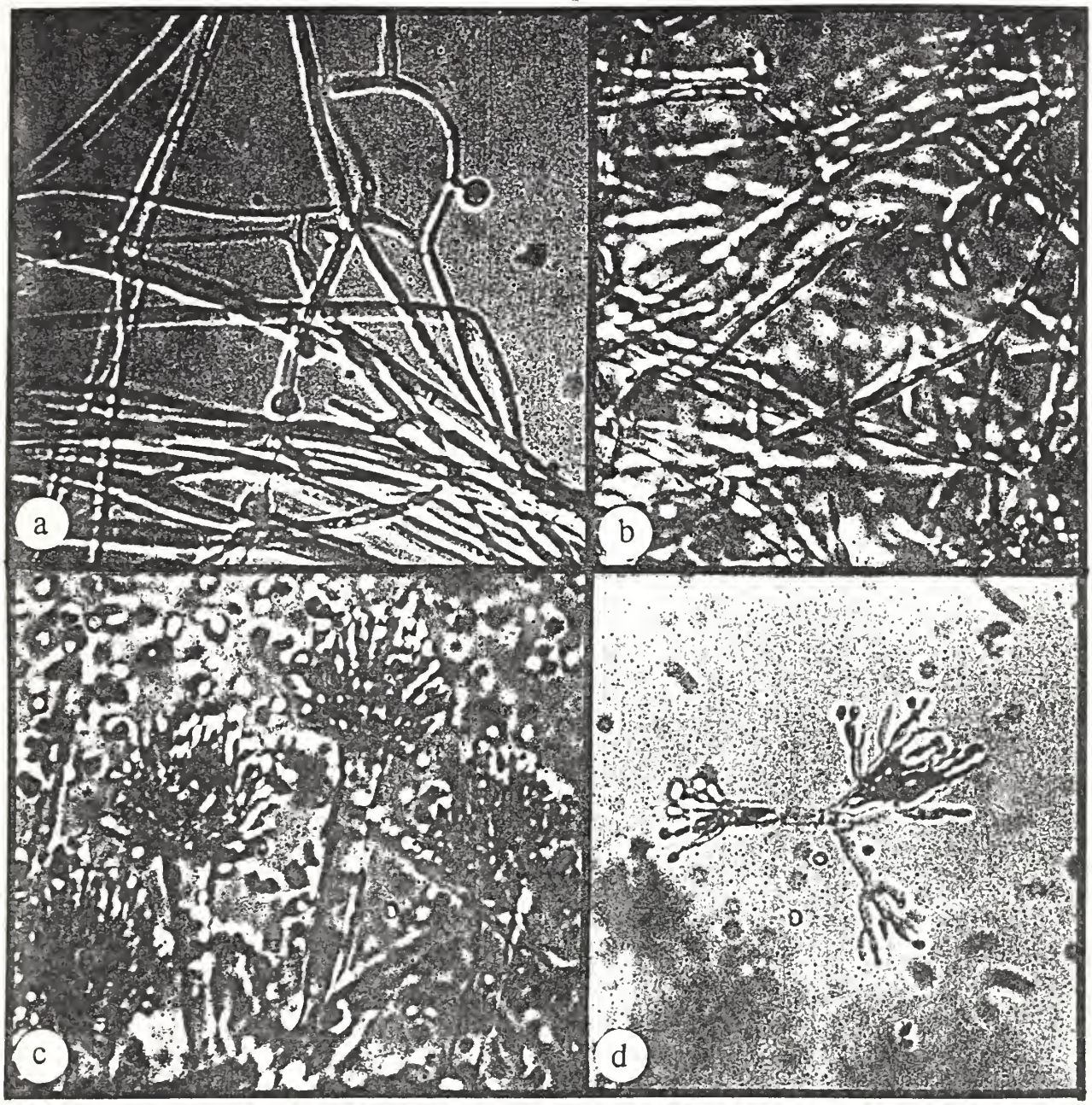

Figure 1a. Lateral globose aleurospore of *Humicola grisea* var. *thermoidea*, a thermophilic Deuteromycetes. 500x in water.

b. Pyriform aleurospore of *Humicola insolens*, a thermophilic Deuteromycetes. 500x in water.

c. Conidial heads of *Aspergillus fumigatus*, a thermotolerant Deuteromycetes. 500x in polyvinyl alcohol mounting medium (PVA).

d. Conidiophores of *Penicillium dupontii*, a thermophilic Deuteromycetes. 500x in PVA.

Table 1. *Fusarium* spp. population levels (means) in the control and solar plots before (June) and after (August) solar heating at Bessey Nursery, 1985.

Treatment	Means * June	August
Control	533.3 b	288.8 b
Solar	1177.8 c	66.6 a

* Means followed by the same letter are not significantly different at $P<0.05$, as determined by the test for equality of means.

Table 2. Most probable number (MPN)* of microorganisms in soil samples from control and solar plots before (June) and after (August) solar heating at Bessey Nursery, 1985.

Incubation Temperature	Soil Sample	June MPN	August MPN
30°C	Control 1	4.3×10^{9}	6.8×10^{6}
	2	8.1×10^{8}	9.3×10^{6}
	3	$>1.6 \times 10^{10}$	4.9×10^{7}
	4	1.6×10^{10}	7.8×10^{6}
	Mean	9.3×10^{9}	1.8×10^{7}
	Solar 1	4.8×10^{8}	0.2×10^{7}
	2	4.3×10^{8}	4.6×10^{7}
	3	6.4×10^{8}	1.7×10^{7}
	4	$>1.6 \times 10^{10}$	3.3×10^{7}
	Mean	4.4×10^{9}	2.5×10^{7}
50°C	Control 1	1.2×10^{7}	$<1.0 \times 10^{5}$
	2	2.1×10^{8}	4.0×10^{5}
	3	4.0×10^{7}	4.0×10^{5}
	4	1.4×10^{6}	$<1.0 \times 10^{5}$
	Mean	6.6×10^{7}	2.5×10^{5}
	Solar 1	2.0×10^{5}	9.3×10^{5}
	2	2.0×10^{5}	4.5×10^{5}
	3	1.4×10^{6}	2.0×10^{5}
	4	2.6×10^{6}	2.0×10^{5}
	Mean	1.1×10^{6}	4.5×10^{5}

* MPN per gram of soil incubated in nutrient broth with 2.5% glucose for two weeks at 30° or 50°.

While population levels (determined by dilutions incubated on MYSA) of thermophilic/thermotolerant fungi remained constant in control soil, solar heating resulted in a 28-fold increase, mostly *Aspergillus fumigatus* (Table 3). The numbers of *A. fumigatus* colonies obscured other microorganisms in solar-heated samples. Although the soil dilutions on YSA resulted in bacteria-covered plates, Actinomycetales which inhibited other bacteria in their immediate vicinity were observed and tallied in the pretreatment and control samples. Population levels of Actinomycetales decreased in control soil, and none were recovered in the solar-heated samples. Because the increases in population levels of the thermophilic/ thermotolerant fungi due to solar heating were so dramatic, no statistical analysis was performed.

Table 3. Propagules per gram of soil of thermophilic/thermotolerant fungi (on MYSA) and Actinomycetales (on YSA) from soil dilutions incubated at 50°C, from control and solar plots before (June) and after (August) solar heating at Bessey Nursery, 1985.

Soil Sample	June		August	
	Fungi	Actinomycetales	Fungi	Actinomycetales
Control 1	330	5400	400	3000
2	0	11,000	350	0
3	670	12,000	350	250
4	0	13,000	75	2300
Mean	250	10,000	290	1400
Solar 1	0	530	44,000	0
2	4600	350	86,000	0
3	2000	3600	65,000	0
4	4300	4600	110,000	0
Mean	2700	2300	76,000	0

Antagonism Tests

Results of the cross-streak and opposing-plugs antagonism tests are given in Table 4. The thermotolerant fungus, *Aspergillus fumigatus* (197), was a strong competitor against all the *Fusarium* isolates. Three bacterial isolates (270, 271, and 272) were effective competitors against two *F. oxysporum* isolates (19 and 26), while all *Fusarium* isolates except *F. oxysporum* (25) were limited by the bacterial isolate (272). This bacterial isolate caused a heavy staining reaction with *F. moniliforme* and limited its growth.

Thermophilic fungi and Actinomycetales were equally competitive with or overgrown by the *Fusarium* isolates. *A. fumigatus* (269) interacted with *F. acuminatum* and *F. oxysporum* (25) to form narrow inhibition zones of no growth. *A. fumigatus* (268) was much less competitive than (269), which out-competed all but *F. oxysporum* (26).

Based on interactions with the microorganisms tested, the order of competitive ability of the *Fusarium* isolates at 30°C on YSA, from most competitive to least, was *F. oxysporum* (25), *F. oxysporum* (19), *F. moniliforme* (29), *F. oxysporum* (26), and *F. acuminatum* (23). *F. acuminatum* did not grow as well in pure culture at 30°C as did the other *Fusarium* isolates. The isolates of *Aspergillus fumigatus* also differed in competitive ability and interaction with the *Fusarium*.

Most fungal pathogens are not thermotolerant and have thermal optima about 25°C. Some thermotolerant microorganisms outgrew *Fusarium* at temperatures as low as 30°C. The competitive ability of thermotolerant microorganisms, and their population increases (especially *Aspergillus fumigatus)* following solar heating, implicate them as playing a role in pathogen limitation in solar-heated soils.

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Table 4. Interactions of *Fusarium* and thermophilic/thermotolerant isolates after two weeks at 30°C. Accession numbers at the Fusarium Research Center (FRC) for five *Fusarium* isolates, and at the American Type Culture Collection (ATCC) for three thermophilic/thermotolerant isolates are given.

Fusarium Isolate	Interactions[a] with Thermophilic/Thermotolerant Isolates F+	F=T	T+
(19) *F. oxysporum* FRC O-1517	Actinomycetales[b] (186,187,189) *Humicola insolens* [b] (198) *H. grisea* var. *thermoidea* [b](196) bacteria[b] (273) ascomycete[c] (256) *Penicillium dupontii* [c] (259) ATCC 60656 deuteromycete[c] (264) deuteromycete[c] (267)	*Aspergillus fumigatus* (268) ATCC 60654	*A. fumigatus* [b] (197) bacteria[b] (270,271,272) *A. fumigatus* [c] (269) ATCC 60655
(23) *F. acuminatum* FRC R-8223	*H. grisea* var. *thermoidea* [b](196) *H. insolens* [b] (198) bacteria[b] (270,271,273) *P. dupontii* [c] (259)	Actinomycetales[b] (186,187,189) ascomycete[c] (256) deuteromycete[c] (264,267) *A. fumigatus* [c] (268)	*A. fumigatus* [b] (197) bacteria[b] (272) *A. fumigatus* [c] (269)
(25) *F. oxysporum* FRC O-1518	Actinomycetales[b] (186,187,189) *H. grisea* var. *thermoidea* [b](196) *H. insolens* [b] (198) bacteria[b] (270,271,272,273) ascomycete[c] (256) *P. dupontii* [c] (259) deuteromycete[c] (264,267) *A. fumigatus* (268)		*A. fumigatus* [b] (197) *A. fumigatus* [c] (269)
(26) *F. oxysporum* FRC O-1519	Actinomycetales[b] (186,187,189) bacteria[b] (273) *A. fumigatus* [c] (268)	ascomycete[c] (256) *P. dupontii* [c] (259) deuteromycete[c] (264,267) *A. fumigatus* [c] (269)	*A. fumigatus* [b] bacteria[b] (270,271,272)
(29) *F. moniliforme* FRC M-3334	Actinomycetales[b] (186,187,189) bacteria[b] (270,271,273) *P. dupontii* [c] (259) *A. fumigatus* [c] (268)	ascomycete[c] (256) deuteromycete[c] (264,267)	*A. fumigatus* [b] (197) bacteria[b] (272) *A. fumigatus* [c] (269)

[a] F+ = *Fusarium* mycelium overgrew thermophile; F=T = *Fusarium* growth stopped at thermophile but without zones of no growth; T+ = thermophile overgrew *Fusarium*.

[b] *Fusarium* plugs adjacent to streak of thermophilic/thermotolerant isolate on yeast starch agar (YSA).

[c] *Fusarium* plug adjacent to plug of thermophilic/thermotolerant isolate on YSA.

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